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Bioactivities of Salam Leaf (*Syzygium polyanthum* (Wight) Walp)

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Abstract. Plants are source of medicinal compounds. Salam leaf or Indonesian bay leaf (*Syzygium polyanthum* (Wight) Walp) from the family of *Myrtaceae* are widely used as herbs and spices for cooking (culinary) and as traditional medicine for diabetes, diarrhea, and hypertension. Indonesian bay leaf contains many compounds including essential oils, tannins, and flavonoids. In this study, 70 % and 96 % ethanol extracts of bay leaf obtained from the garden in Research Center for Science and Technology (PUSPIPTEK), Serpong, Tangerang Selatan, Indonesia were tested *in vitro* according to α-glucosidase inhibition method to determine antidiabetic activity and to scavenge DPPH free radicals in order to determine antioxidant activity and toxicity on Brine Shrimp Lethality Test (BSLT). The results showed that extraction of Indonesian bay leaf by using 70 % and 96 % ethanol extracts have anti diabetic activity (97.37 % and 95.51 % α-glucosidase inhibition) and antioxidants activities (89.66 % and 69.18 % DPPH inhibition), respectively and have very low toxic effects on brine shrimp larvae (LD50 > 1,000 μg/mL).

Keywords: Bay leaf (*Syzygium polyanthum* (Wight) Walp.), bioactivities, DPPH

INTRODUCTION

Indonesia is one of the countries having high biodiversity in the world. This high biodiversity can be used as a great resource, in which the global challenge and opportunity tend to follow healthy life model and has owned trust in cultures around the world that natural medicinal is safer compared to synthetic medicinal [1]. Using natural resources as medicinal material is a culture which had been carried out by community through heritage since centuries ago. On the other hand, security and outcome on healthy aspect are not totally supported by suitable research result yet [2]. Invention on medicinal plants from natural resources need long period, however in searching new medicinal is still an important option. A lot of medicinal used clinically are achieved from medicinal plant and their derivatives [3].

Salam plant (*Syzygium polyanthum* (Wight) Walp) is one of the Indonesia huge biodiversity originating from family *Myrtaceae*. Other name is *Eugeniea polyantha* Wight, *E. nitida* Duthie, and *E. balsamea* Ridley [4] and well-known by the community as cooking (culinary) ingredients. Bay leaf tree grows wild in the forest and mountain in the Western part of the South East Asian peninsular (Myanmar, Thailand to Malaysia) and in Western Indonesia. The plant is cultivated in yard and in the neighborhood. In Indonesia, bay leaves have many names, such as meselengan, ubar serai (Sumatera), Salam, gowok (Sunda), manting (Jawa), and kastolam (Kangean) [5, 6]. Salam leaf in Indonesia are used to treat diabetes, anti-obesity, hyperuricemia, arthritis and antidiarrheal [7-11]. The Salam leaf contains tannin, essential oil (salamol eugenol), flavonoid (quercetin, quercitrin, myricetin, myricitrin), sesquiterpenes, triterpenoid, phenol, steroid, citral, lactone, saponin, and carbohydrate [12]. Polyphenols (phenolic acids and

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flavonoids), alkaloids, steroids or triterpenoids (saponins), and anthraquinones are some compounds that can function as antioxidants [13, 14]. These compounds might have relation with the use of Salam leaf. Antioxidants are chemical compounds that can neutralize free radical agents which work by donating electrons to achieve a stable form. Human requires antioxidants from outside the body in addition to the antioxidants that are in their own body to achieve balance condition [15]. Enzymes and non-enzymes are antioxidant compounds in the body, but in the case of oxidative stress is unable to resist oxidants. Therefore, it is necessary to have exogenous antioxidant compounds [16]. Antioxidant administration in the form of vitamins can reduce oxidative stress for both chronic and acute DM-1 sufferers [17].

In this experiment activity, Salam leaf (*Syzygium polyanthum* (Wight) Walp) was collected from plantation field in PUSPIPTEK cluster, Serpong, South Tangerang, Indonesia. It was tested for its bioactivity according to *in vitro* through inhibiting α-glucosidase in order to determine activity of antidiabetic and to scavenge DPPH free radical in order to determine activity of antioxidant and toxicity on Brine Shrimp Lethality Test (BSLT).

MATERIALS AND METHOD

Plant Materials

Plant materials *Syzygium polyanthum* (Wight) Walp leaves were subsequently collected from Kebun Bumbu (Spices Garden) located in Kawasan PUSPIPTEK Serpong, South Tangerang, sorted and dried in oven at 50 °C, reduced size by means of blender, sieved via a 20 mesh sieving in order to yield a uniform size powder.

Preparation of Extract

1.6 g bay leaf were macerated by using 70 % ethanol (20 mL) and 96 % ethanol (20 mL) for 3 days, filtered by filtration paper (Whatman No. 2), and concentrated by means of rotary evaporator at 50 ℃ and speed 150 rpm.

Phytochemical Screening

Qualitative analysis on alkaloid, flavonoid, tannin, terpenoid, steroid, and saponin was performed according to method described in [18]. In brief, contents of alkaloid, flavonoid, tannin, terpenoid/steroid, and saponin were specified by separating reaction and recording the change of color. Besides, quantitative analysis was also conducted on concentrations of total phenol and total flavonoid. Total phenol concentration in extract was determined with Folin–Ciocalteu method achieved as equivalent to gallic acid, meanwhile total flavonoid concentration was achieved as equivalent to quercetin according to method of aluminum chloride colorimetric assay [19].

In Vitro Antioxidants Assay

Antioxidant activity test from extract on free radical was performed according to method of *"DPPH free scavenging activity"* Yen and Chen modified [20, 21]. A number of sample was dissolved in methanol (10–200 µg in concentration), reacted with one 1 mL DPPH (1 mM in methanol), incubated at room temperature for 30 minutes, and measured for its absorbance at wavelength 515 nm. Antioxidant Activity was calculated as percentage inhibition on DPPH (percentage of *"scavenging effect"),* due to difference in adsorption between blank and sample.

In Vitro Antidiabetic Assay

Inhibition activity test on α-glucoside was conducted based on the procedure from Kim [22] with small alteration. Solutions of *p-nitrophenyl-α-D-glucopyranoside* 5 mM (25 µL) and 0.1 M buffer phosphate pH 7 (50 µL) were inserted into microplate, which had been filled with 5 µL sample solution in DMSO to make final concentration of 100 µg/mL. Mixture of these solutions were pre incubated at 37 °C for 5 minutes, enzymatic reaction was started by

adding 25 µL solution of α-glucosidase (0.063 units), and incubated for 15 minutes. Reaction was discontinued by adding 95 µL Na_2CO_3 solution (0.2 M). Enzymatic activity was determined by observing p-nitrophenol formed by means of spectrophotometer at wavelength 400 nm.

Brine Shrimp Lethality Test (BSLT)

Toxicity test was carried out according to general bioassay method in order to determine toxicity activity in *in vitro,* as described by Meyer et al. [23]. Toxicity activity of sample with concentrations 10, 100, 500 and 1,000 µL/mL were calculated after 24 hours of breeding salinity shrimp larvae (*Artemia salina).* Samples showed a very low toxic effect on brine shrimp larvae if LC50 is smaller than 1.000 µg/mL (LC50 < 1,000 µg/mL).

RESULTS AND DISCUSSION

In the preparation of extracts, the size of bay leaf simplicia was reduced to become powder that passes through 20 mesh sieve. The size reduction was conducted to maximize the extraction process because the small particle size produces a large surface area so that the contact of the powder with the extraction solvent increases, consequently the greater the ability of the solvent to extract the active compounds in leaf simplicia [24] .

This study used ethanol as extraction solvent because bay leaf itself are used for food ingredients and the solvents allowed to be used in food ingredients are ethanol, ethyl acetate and acetone alone [25]. Another reason for using ethanol as a solvent is because it is classified as a solvent polar which means that this solvent can dissolve in water and will extract most of the ionic compounds from the bay leaf [26].

Table 1 shows that the chemical content of bay leaf are alkaloids, flavonoids, saponins, tannins, quinones and terpenoids. These results are in line with previous studies which 96 % ethanol solvents is used. It was reported that the chemical content of bay leaf from Central Java are phenolic, flavonoids, terpenoids, saponins and alkaloids [27].

Table 2 shows total phenol and total flavonoids content of bay leaf extracts. When compared with other studies reported by Ismiyati [27] total phenol content of this study was higher but the total flavonoids content were lower than reported in those studies. The differences might be due to the different origin of the samples as also observed in study on pegagan (*Centella asiatica)* extracts [21].

Different levels of the chemical content as shown in (Table 1 and Table 2) shows that the polarity of the solvent and the different origin of samples have effect on the chemical content of the extracts obtained. Similar observation was also reported in study on pegagan (*Centella asiatica*) extracts [21]. The presence of water in 70 % EtOH might affect the solubility of certain compounds which have low solubility in 96 % EtOH.

TABLE 1. Phytochemical screening results of 70 % and 96 % EtOH bay leaf extracts.

Phytocemical test	EtOH 70 %	EtOH 96 %
Alkaloid		
Bouchardat	++	+
Meyer	-	-
Dragendrof	+++	++
Flavanoid	++	+
Saponin	+	+++
Tanin	++	++
Quinon	+	+
Terpenoid	+	++

Note:
- \- shows the absence of phytochemicals
- \+ shows the presence of phytochemicals
- ++ shows the presence of abundant phytochemicals
- +++ shows the presence of highly abundant phytochemicals

TABLE 2. Total phenol and total flavonoid content of 70 % and 96 % EtOH bay leaf extracts.

Solvent	Total phenol µg gallic acid equivalent/mg DW	Total flavanoid µg quercetin equivalent /mg DW
70 % EtOH	445.9	392.6
96 % EtOH	265.1	278.7

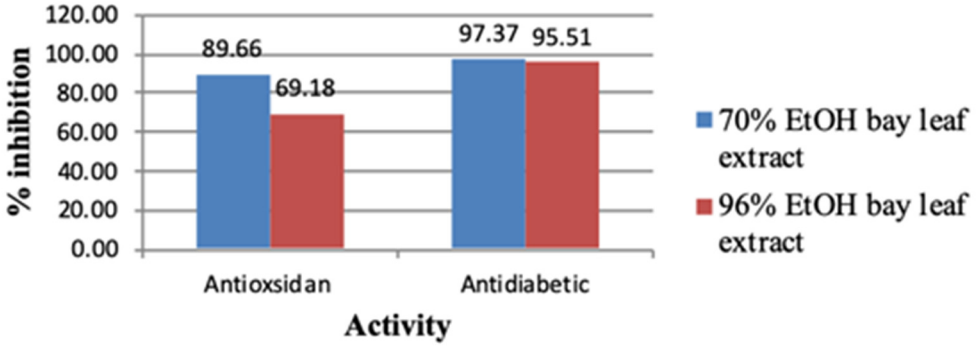

FIGURE 1. Antioxidant and antidiabetic activity of 70 % and 96 % ethanol bay leaf extract.

TABLE 3. BSLT results of 70 % and 96 % ethanol bay leaf extract.

Solvent	LC50
70 %	707.945 µg/mL
96 %	977.237 µg/mL

Figure 1 shows the results of antioxidant and antidiabetic activitities of 70 % and 96 % bay leaf extracts. In both assays the results of 70 % ethanol extract of bay leaf were higher than 96 % ethanol extract of bay leaf. Previous research on 95 % ethanol bay fruit extract showed 86–90 % which similar to our results with bay leaf extracts [28].

In vitro toxicity assay of 70 % and 96 % ethanol bay leaf extract was conducted with the Brine Shrimp Lethality Test (BSLT) method to determine the toxicity value using Lethal Concentration (LC50). The value of LC50 is the number of levels that cause death of 50 % of test animals in a certain period of time. The LC50 does not focus on the specific organ damage but on the total mortality of the test animal itself, so that the LC50 value is used in the short-term test. LC50 is used to calculate the death rate of artemia deaths given the uncomplicated digestion structure and high sensitivity [29]. Active compounds will produce high mortality, compounds with LC50 ≤ 1000 µg/mL can be categorized as compounds that have potential as toxic compounds, the smaller the LC50 value, the compound is said to be more active/toxic [23].

Table 3 shows the BSLT results 70 % and 96 % ethanol bay leaf extract. The results showed that the higher the concentration, the more the death of shrimp larvae showed that the higher the toxic properties of the compounds in the extract, LC50 value of 70 % ethanol extract of bay leaf is 707.945 µg/mL and LC50 ethanol extract 96 % bay leaf 977.237 µg/mL, both were non-toxic because LC50 ≥ 1000 µg/mL.

CONCLUSION

Extraction of bay leaf using 70 % and 96 % EtOH shows significant results in *in vitro* antioxidant and antidiabetic activities and showed only low toxicity to brine shrimp. These results suggest that these extracts have potential to be used as herbal for diabetes treatment.

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